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Observations on Some of the Mycelial Growth and Pigmentation Characteristics of *Cordyceps militaris* Isolates

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Characteristic growth patterns of *Cordyceps militaris* isolates on various media, under varying light conditions and at varying incubation periods were examined. Light was found to be the most critical single factor in determining the density, texture, and pigmentation of the mycelial culture of the fungus. However, under the light condition, the degree of pigmentation and mycelial density were found to be affected by the incubation period and type of medium. Irrespective of the variations in medium type or incubation period, there was no pigmentation of the mycelium under dark condition. Radial growth of the mycelium was faster under dark incubation rather than under light incubation. Abundant mycelial density and darkest pigmentation of *C. militaris* isolates were produced in nutritionally rich media like SDAY, SMAY and CZYA, suggesting that these media may fulfill all the requirements for vegetative growth of the fungus. Growth characteristics of *C. militaris* isolates could be easily observed by the simple agar culture method, which would be useful to characterize the phenotypic characteristics of large number of pure cultures of the fungus under given conditions of growth factors such as medium, light and temperature.

KEYWORDS: Colony pigmentation, *Cordyceps militaris*, Light and dark cultures, Mycelial growth, Rich and poor media

Studies on *in vitro* mycelial growth of *Cordyceps* and other entomopathogenic fungi are of high interest due to their high medicinal values and their potential as biocontrol agents of pests. *Cordyceps militaris*, type species of the genus *Cordyceps*, attracted the attention of de Bary more than a hundred years ago, who studied its mycelial growth by infecting larva of *Sphinx euphorbiae* with ascospores of the fungus and by growing them in moist sand (de Bary, 1867, 1887). Since then, mycelial growth characteristics of *C. militaris* have been studied on various agar media (Pettit, 1895; Petch, 1936; Shanor, 1936; Brown and Smith, 1957; Gams, 1971; Wang, 1989; Sung *et al*., 1993, 2002). Various carbohydrate sources such as glycerol, glucose, fructose, galactose, mannitol, maltose, sucrose, cellobiose, glycogen, starch, etc. have been found to result in vigorous mycelial growth of *C. militaris* in agar culture (Basith and Madelin, 1968). Similarly, both inorganic nitrogen sources such as nitrate and ammonium, and organic nitrogen sources such as L-asparagine and peptone have been reported to be readily utilized by *C. militaris* (Basith and Madelin, 1968).

Culture media play important roles for the optimum mycelial growth of different fungal species. In case of *Cordyceps* species, different types of media are used for isolation, identification, preservation and *in vitro* culture, with less uniformity in medium composition and incubation period. Hence, it is of scientific as well as applied interest to study growth characteristics of *C. militaris* in a range of nutritionally poor to rich agar media at different intervals of incubation period. As a result, preference of certain media over others can be made for fulfilling the growth requirements of *C. militaris* during its regular culture and sub-culture, long-term preservation, *in vitro* stroma production as well as for the production of secondary metabolites in culture.

In addition, medium experiments can be helpful to describe phenotypic characters of pure isolates of *C. militaris* in defined growth conditions of medium, light, temperature, pH, etc. The present study aims at describing colony growth characteristics of *C. militaris* on various agar media at different incubation periods in both light and dark conditions.

Materials and Methods

Fungal isolates. Five mono-ascospore isolates, EFCC C-7159-1, -2, -3, -4 and -5, isolated from *C. militaris* specimen EFCC C-7159, were examined for their growth characteristics in agar media. For the isolation of mono-ascospores, fresh stroma of *C. militaris* specimen EFCC C-7159 was placed over 2% water agar (WA) and was regularly observed for the discharge of ascospores. Five mono-ascospores were picked from WA using a Zeiss dissecting microscope Stemi SV11 with the help of a sterile insect pin, inoculated in new WA plates and grown for 4 weeks at 25°C under the white fluorescent light, follow-

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ing the method described by Paterson and Bridge (1994). The specimen EFCC C-7159, grown on a pupa host, was originally collected from Mai Mt., Jeonla-Do, Korea in July 2001 and has been preserved in the Entomopathogenic Fungal Culture Collection (EFCC), Kangwon National University, Korea.

Culture media. Twenty-two different types of agar media, categorized into poor, general purpose and rich on the basis of literature survey, were employed for the observation of colony growth characteristics of the five isolates of *C. militaris*, EFCC C-7159-1, -2, -3, -4 and -5 (Table 1). Media compositions (Table 2) were adopted from different sources (Tuite, 1969; Stockdale, 1971; Stevens, 1981; Johnston and Booth, 1983; Atlas, 1993; Sung *et al*., 1993). pH of YpSs and MCM was adjusted to 7.0, whereas that of CDA and CZYA was adjusted to 7.3. But, pH of the rest of the media was adjusted in between 5.5~6.0, as shown in Table 2. pH of all the media was adjusted before autoclaving. The media, after autoclaving, were poured into 87 × 15 mm sterile plastic Petri dishes (15~20 *ml* per plate). Amount of agar varied from medium to medium (Table 2).

Growth conditions and observation. Mycelial discs (4 mm) were cut from colonies of all the five isolates of *C. militaris* grown in WA, and were inoculated in each of twenty-two different agar media including WA (Table 1). Inoculated plates were incubated separately under both light and dark conditions in triplet at 25°C. Colony growth characteristics such as mycelial density, mycelial texture and colony pigmentations of all the isolates were recorded each week for four subsequent weeks. But, colony diameter could not be recorded after three weeks of incubation as most of the colonies completely covered the agar plates. Mycelial density was visually classified into poor (+), moderate (++) and abundant (+++) depending upon the density of aerial mycelia. Extremely poor mycelial density was denoted as (−). Similarly, mycelial texture was categorized into flat (F), semi-cottony (SC) and cottony (C). In case of dark incubation, agar plates once exposed to the light for the examination were discarded instantly. Colony pigmentation was described based on the method of Kornerup and Wanscher (1978).

Results and Discussion

Mycelial density and texture. In this study, agar media were used to assess different growth characteristics of *C. militaris*, as the method was easy to monitor the variations amongst the isolates. Also, it was possible to observe changes in the growth patterns at different time intervals. Solid-state medium might be more realistic to study the growth characteristics of fungal species such as *Cordyceps*, which are non-aquatic in natural habitat. Our previous experience has shown that liquid culture does not

Table 1. Categories of different media used in the present study

Category	Full names of media along with their abbreviations	Reference
Poor media	Water agar (WA)	Stevens (1981)
	Potato-Carrot agar (PCA)	Tuite (1969)
General purpose media	Malt agar (MA)	Johnston and Booth (1983)
	Cornmeal agar (CMA)	Stevens (1981)
	Oatmeal agar (OA)	Stevens (1981)
	Malt-extract agar (MEA)	Atlas (1993)
	Malt-Yeast agar (MYA)	Tuite (1969)
	V8 Juice agar (V8)	Johnston and Booth (1983)
	Hamada agar (HA)	Sung *et al.* (1993)
	Schizophyllum (Mushroom) Genetics Minimal Medium (MM)	Stevens (1981)
	Emerson YpSs agar (YpSs)	Tuite (1969)
	Czapek-Dox agar (CDA)	Stevens (1981)
	Yeast-extract Malt-extract Peptone Dextrose agar (YMA)	Stevens (1981)
	Ebiose Sucrose agar (ES)	Sung *et al.* (1993)
Rich media	Potato-Dextrose agar (PDA)	Johnston and Booth (1983)
	Martin's Peptone Dextrose agar (MPDA)	Tuite (1969)
	Schizophyllum (Mushroom) Genetics Complete Medium plus Yeast Extract (MCM)	Stevens (1981)
	Sabouraud Maltose agar (SMA)	Johnston and Booth (1983)
	Sabouraud Dextrose agar (SDA)	Johnston and Booth (1983)
	Czapek Yeast Extract agar (CZYA)	Stevens (1981)
	Sabouraud Maltose agar plus Yeast Extract (SMAY)	Stockdale (1971)
	Sabouraud Dextrose agar plus Yeast Extract (SDAY)	Stockdale (1971)

Table 2. Composition and concentration (g/*l*) of culture media used in the present study

Media[a] / Components	WA	MA	CMA	PCA	MEA	V8	ES	OA	MYA	MM	YpSs	MPDA	PDA	MCM	SMA	SDA	YMA	HA	CDA	CZYA	SMAY	SDAY
Dextrose					20				4	20		10	20	20		40	10	20				40
Malt extract		20			20				10								3					
Maltose															40						40	
Sucrose							20												30	30		
Starch											15											
Potato				20									200									
Corn meal			50																			
Oatmeal flake								30														
Carrot				20																		
Peptone					1							5		2	10	10	5				10	10
Yeast extract									4		4			2			3	3		5	10	10
DL-asparagine										2												
$NaNO_3$																			3	3		
Ebiose							5											5				
V-8 Juice						200																
$MgSO_4 \cdot 7H_2O$										0.5	0.5	0.5		0.5					0.5	0.5		
KCl																			0.5	0.5		
$FeSO_4 \cdot 7H_2O$																			0.01	0.01		
KH_2PO_4										0.46		1		0.46								
K_2HPO_4										1	1			1					1	1		
Thiamine·HCl										120 µg												
Hyponex																		3				
pH		6.0	6.0	6.0	5.5	6.0	6.0	6.0	6.0	5.6	7.0	6.0	6.0	7.0	5.6	5.6	6.0	5.5	7.3	7.3	5.6	5.6
Agar	20	20	15	20	20	20	20	20	15	20	20	20	20	20	15	15	20	15	20	20	15	15

[a]Refer to Table 1 for the abbreviations of the media.

Table 3. Mycelial growth characteristics of *Cordyceps militaris* EFCC C-7159 isolates on various media and at different incubation periods under light condition

Medium	1st wk.		2nd wk.		3rd wk.		4th wk.	
	Mycelial density	Mycelial texture	Mycelial density	Mycelial texture	Mycelial density	Mycelial texture	Mycelial density	Mycelial texture
WA	–[a]	F[b]	–	F	–	F	–	F
MA	+	F	+	F	+	F	+	F
CMA	+	F	+	F	+	F	+	F
PCA	+, ++	F	+, ++	F	+	F	+	F
MEA	+	F	+, ++	F	+, ++	F	+, ++	F
V8	++, +++	F	+, ++	F	+, ++	F	+, ++	F
ES	++	F	++	F	++	F	++	F
OA	++, +++	F	++, +++	F	++	F	++	F
MYA	+++	F	++, +++	F	++, +++	F	++	F
MM	+, +++	F	++, +++	F	++, +++	F	++, +++	F to SC
YpSs	++, +++	F	++, +++	F	++, +++	F	++, +++	F
MPDA	++, +++	F	++, +++	F	++, +++	F	++, +++	F to SC
PDA	++, +++	F	++, +++	F	++, +++	F	++, +++	F
MCM	++, +++	F	++, +++	F	++, +++	F	++, +++	F to SC
SMA	+++	F	++, +++	F	++, +++	F	++, +++	F to C
SDA	+++	F	++, +++	F	++, +++	F	++, +++	F to C
YMA	+++	F to SC	++, +++	F	++, +++	F	++, +++	F
HA	+++	F	++, +++	F	++, +++	F	++, +++	F
CDA	+	F	++, +++	F	++, +++	F	+++	F to C
CZYA	+++	F to C	++, +++	F to C	++, +++	F to C	+++	F to C
SMAY	+++	F to C	+++	F to C	+++	F to C	+++	F to SC
SDAY	+++	F to C	+++	F to C	+++	F to C	+++	F to SC

[a]–, extremely poor; + poor; ++ moderate; +++ abundant.
[b]F, flat; SC, semi-cottony; C, cottony.

give characteristic features in different isolates of *C. militaris* by culturing in different media. In this study, the exact amount of mycelial growth could not be measured. Hence, most of the growth characteristics of *C. militaris* have been defined in comparative descriptive terms.

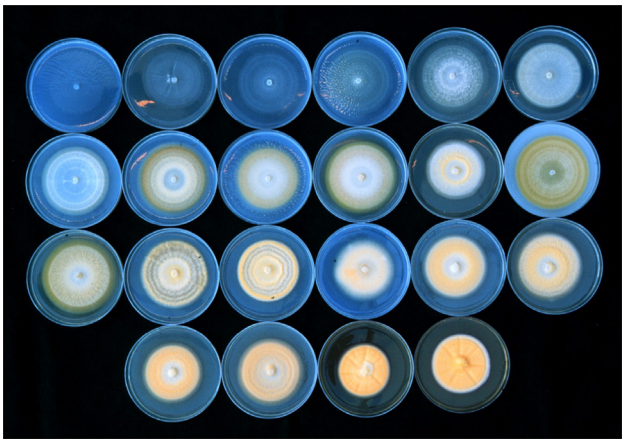

Fig. 1. Colony pigmentations of *Cordyceps militaris* isolate EFCC C-7159-3 after three weeks of light incubation on different agar media. Medium series (from top left to bottom right): WA, MA, CMA, PCA, V8, MEA, ES, MPDA, MMM, PDA, MYA, OA, YpSs, SMA, SDA, CDA, MCM, YMA, CDYA, HM, SMAY and SDAY.

Mycelial density of all the five isolates of *C. militaris* in light incubation was always observed to be extremely poor in WA, poor in MA and CMA, while poor to moderate in PCA, MEA, V8 and ES (Table 3, Fig. 1). But, the mycelial density varied from moderate to abundant in the rest of media, except for SDAY and SMAY, which always developed abundant mycelial density (Table 3, Fig. 1). Three of the media, MA, CMA and PCA consisted mostly of carbohydrate sources, as a consequence of which poor mycelial density was observed in these media. Similarly, mycelial density was poor to moderate in MEA, V8 and ES, which contained higher amounts of carbon and nitrogen sources than MA, CMA and PCA. The rest of the media, however, contained highest concentrations of carbon and nitrogen sources (both organic as well as inorganic in case of nitrogen source) including inorganic mineral salts (in case of few), hence, producing moderate to abundant mycelial density. The media containing extra supplements of mineral salts like $MgSO_4.7H_2O$, KCl, $FeSO_4$. $7H_2O$, KH_2PO_4 and K_2HPO_4 such as MM, YpSs, MPDA, MCM, CDA and CZYA, however, did not produce the highest mycelial density amongst all the media.

In our study, it could be observed that vegetative mycelium of *C. militaris* could grow in a wide range of media, including WA, suggesting that it is a facultative phag-

otrophic fungus in nutrition habit. Entomopathogenic fungi have been found to survive well with restricted growth in soil for a long period of time without infecting its host (Kamata, 2000). Mycelial density exhibited some variations depending on the incubation period in few media. For instance, in CDA, the mycelial density was poor during the first week but had a rapid and abundant growth in the fourth week. Similarly, the density was moderate to abundant till the first and second week in V8 and OA, respectively, but could not produce abundant density later on.

Under light incubation, mycelial texture of all the five isolates was flat in most of the media, except in CZYA, SDAY and SMAY which resulted in both flat and convex types of textures from the early incubation period (Table 3, Fig. 1). Some media, such as MM, MPDA, MCM, SMA, SDA and CDA, however, appeared to induce cottony type of texture during late incubation periods. Fungal mycelium can adopt flat or cottony type of mycelial growth, on the basis of availability of nutrition, although it differs from species to species and strain to strain (Carlile, 1995). In the present study, colonies were found to be mostly flat on nutritionally less rich media, while flat to cottony textures were observed in highly rich media like CZYA, SDAY and SMAY.

Under dark incubation, the mycelial density was extremely poor in WA and poor in MA, whereas poor to moderate in CMA and PCA, moderate to abundant in MEA, V8, ES, OA, MM, YpSs and CDA and abundant in the rest of the media throughout the incubation period (Fig. 2). Surprisingly, all the isolates of *C. militaris* produced cottony type of mycelial texture in from the beginning all the media under dark incubation (Fig. 2).

Fig. 2. No colony pigmentations of *Cordyceps militaris* isolate EFCC C-7159-3 after three weeks of dark incubation on any of the agar media. Medium series (from top left to bottom right): WA, MA, CMA, MEA, OA, PCA, V8, ES, CDA, YpSs, MYA, HM, PDA, MCM, CDYA, MMM, MPDA, SMA, SDA, SDAY and SMAY. Note: YMA is missing.

Mycelial growth characteristics showed less difference amongst different media under dark incubation when compared to those grown under light incubation. Although mycelial biomass could not be exactly measured in this study, but it could be observed that dark incubation led to more rapid radial growth when compared to the light incubation. Light might have exerted some degree of inhibitory effects on mycelial growth rate of *C. militaris*.

Colony pigmentation. Various degrees of colony pigmentations were produced by incubating all isolates of *C. militaris* in all media in light, except WA, which produced no pigmentation. No pigmentation was, however, produced by all the five isolates in dark incubation, irrespective of medium type and incubation period (Fig. 2). After three weeks of incubation under light, MA and CMA produced the least pigmentation in all isolates, followed by PCA and MEA. In general, yellowish white (2A2, 3A2) pigmentations were produced in MA and CMA, and the remaining media produced pigmentations ranging from yellowish white (2A2, 3A2) to light orange (5A4, 5) (Table 4). CZYA, SMAY and SDAY produced

Table 4. Colony pigmentation of *Cordyceps militaris* EFCC C-7159 isolates on various media after three weeks of incubation under light condition

Medium	Isolates				
	C-7159-1	C-7159-2	C-7159-3	C-7159-4	C-7159-5
WA	C[a]	C	C	C	C
MA	W	W	W	YW	YW
CMA	W	W	W	YW	YW
PCA	YW	YW	YW	GY	PY
MEA	YW	YW	YW	PO	PO
V8	YW	YW	DY	PY	LY
ES	W	YW	PY	LY	LY
OA	YW	LY	LY	LY	LY
MYA	YW	YW	LY	PO	LO
MM	YW	PY	LY	PO	LO
YpSs	YW	PY	LY	LY	LY
MPDA	YW	LY	LY	LO	LO
PDA	YW	YW	LY	PY	LY
MCM	YW	YW	LY	PY	LO
SMA	YW	YW	LY	LO	LO
SDA	YW	YW	LY	LO	LO
YMA	YW	PY	LY	PO	LO
HA	YW	YW	LO	PO	LO
CDA	YW	LY	LY	PY	LY
CZYA	PY	PY	LO	YO	LO
SMAY	PY	PY	LO	LY	LO
SDAY	PY	PY	LO	LY	LO

[a]Pigmentation descriptions acc. to Kornerup and Wanscher (1978): C, colorless; W, white (1A1); YW, yellowish white (2A2, 3A2); PY, pale yellow (2A3, 3A3); LY, light yellow (3A4, 5); DY, dull yellow (3B3); GY, greyish yellow (3B4); PO, pale orange (5A3); YO, yellowish orange (4A6, 7); LO, light orange (5A4, 5).

the darkest pigmentations of all the media. These results revealed that nutrition source was the main factor responsible for the degree of pigmentation in *C. militaris* under light incubation. However, the exact nutrient factor responsible for the induction of pigmentation in *C. militaris* could not be known, although observations showed that peptone and yeast extract were the main components in inducing pigmentation.

Media without peptone or yeast extract or other organic nitrogen sources produced lighter pigmentations than the media containing them. The specific role of yeast extract on pigmentation of *C. militaris* was evaluated by observing the difference in pigmentation between CDA and CZYA. Besides, a single amino acid, DL-asparagine, was found to be as efficient as peptone or yeast extract for inducing pigmentation, as revealed by production of similar types of pigmentations on MM and MCM.

According to our observations, pigmentation of *C. militaris* also varied from isolate to isolate. Isolates EFCC C-7159-1 and EFCC C-7159-2 produced yellowish white (2A2, 3A2) to light yellow (3A4, 5) pigmentations (data not shown), whereas the remaining isolates EFCC C-7159-3 (Fig. 1), EFCC C-7159-4 and EFCC C-7159-5 (data not shown) produced yellowish white (2A2, 3A2) to light orange (5A4, 5) pigmentations in various media after three weeks of light incubation (Table 4). Individually, isolate EFCC C-7159-1 produced the lightest pigmentations, mostly yellowish white (2A2, 3A2), while isolate EFCC C-7159-5 produced the darkest pigmentations, mostly light orange (5A4, 5) (Table 4), showing clear difference in the degree of their pigmentations. The isolates of *C. militaris* could be differentiated from each other based on the degree of their pigmentation, which was more on rich media than on poor media under light incubation.

Incubation period was another important factor for the development of pigmentation in *C. militaris* under light incubation (Table 5). In most of the media, pigmentation was most pronounced after three weeks of incubation when compared to early periods, but tended to fade after four weeks in some media (Table 5). The most remarking change in pigmentation was observed on CDA, which produced no pigmentation or only yellowish white (2A2, 3A2) pigmentation after one week but induced light yellow (3A4, 5) pigmentation from second week onwards along with the formation of pale yellow (2A3) margins. However, on few media such as MA, CMA, ES, OA, MPDA, PDA, SMA, SDA, incubation period had no

Table 5. Colony pigmentation of *Cordyceps militaris* EFCC C-7159 isolates on various media and at different incubation periods under light condition

Medium	Colony pigmentation at different incubation periods							
	1st wk.		2nd wk.		3rd wk.		4th wk.	
	Center	Margin	Center	Margin	Center	Margin	Center	Margin
WA	C[a]	C	C	C	C	C	C	C
MA	W to YW	W	W to YW	W	W to YW	W	W to YW	W
CMA	W to YW	W	W to YW	W	W to YW	W	W to YW	W
PCA	W to PY	W	YW to LY	W	YW to GY	W	W to GY	W
MEA	W to PY	W	YW to LY	W	YW to PO	W	W to GY	W to LY
V8	W to PY	W	YW to LY	W	YW to LY	W	W to GY	W
ES	W to LY	W to YW	W to LY	W	W to LY	W	W to LY	W
OA	YW to LY	W	YW to LY	W	YW to LY	W	YW to LY	W to Y
MYA	W to OW	W	YW to PO	W	YW to LO	W	YW to PO	W
MM	YW to PY	W	YW to LY	W	YW to LO	W	YW to LO	W
YpSs	YW to LY	YW to PY	YW to YO	W to PY	YW to LY	W to PY	YW to MY	W to LY
MPDA	YW to LO	W	YW to LO	W to LO	YW to LO	W to YO	YW to LO	W
PDA	YW to LY	W to PY	YW to LY	PY to LO	YW to LY	PY to Y	YW to LY	W to MY
MCM	YW to LY	W	YW to LO	W	YW to LO	W	YW to LY	W
SMA	YW to LO	W	YW to LO	W	YW to LO	W	YW to LO	W
SDA	YW to LO	W	YW to LO	W	YW to LO	W	YW to LO	W
YMA	YW to PO	W	YW to LO	W	YW to LO	W	YW to PO	W
HA	YW to PO	W to YW	YW to LO	W to YW	YW to LO	W to PY	YW to LY	W
CDA	W to YW	W	YW to LY	W to PY	YW to LY	W to PY	YW to LY	W to PY
CZYA	YW to OW	W	PY to LO	W	PY to LO	W	PY to YO	W
SMAY	YW to OW	W	PY to LO	W	PY to LO	W	PY to LO	W
SDAY	W to OW	W	PY to LO	W	PY to LO	W	PY to LO	W

[a]Pigmentation descriptions acc. to Kornerup and Wanscher (1978): C, colorless; W, white (1A1); YW, yellowish white (2A2, 3A2); PY, pale yellow (2A3, 3A3); LY, light yellow (3A4, 5); MY, mustard yellow (3B6); DY, dull yellow (3B3); GY, greyish yellow (3B4); Y, yellow (3A6, 7); OW, orange white (5A2); PO, pale orange (5A3); YO, yellowish orange (4A6, 7); LO, light orange (5A4, 5).

effect on pigmentation production. Few media such as YpSs, MPDA, PDA, HA, CDA produced characteristic pigmentations on colony margins.

It is well known that fungal mycelia produce different types of pigmentations, when exposed to light (Harding and Mitchell, 1968). In our study, *C. militaris* also produced pigmentations under light incubation, but not under dark incubation. The observations also suggested that the mycelium, not the conidia, produced pigmentations in *C. militaris* isolates as suggested by Gams (1971), since no isolate produced white colonies with pigmented spores. Deep pigmentations during late incubation periods of *C. militaris* could be due to different phases of photoinduced carotenogenesis such as light reaction, protein synthesis and accumulation of carotenoid pigments (Harding and Shropshire, 1980). Friederichsen and Engel (1958) reported carotenoid as the compound, which produces orange color of *C. militaris*.

Besides *Cordyceps*, several studies on chemical analysis of yellow or red pigments of another entomopathogen genus *Beauveria* have been reported (El-Basyouni *et al*., 1968; Wat *et al*., 1977; Wraight *et al*., 1998). Fungal pigmentation has been related to pathogenicity in some pathogenic fungi. Pathogenic fungal isolates producing little or no melanin have been found to be less virulent or pathogenic when compared to those producing high amounts of melanin (Jacobson, 2000). But, yet no association of carotenogenesis with pathogenecity has been reported in pathogenic fungi (Geis and Szaniszlo, 1984). Rather, nutrition and pharmacological values of carotenoid pigmentations such as antitumor agents in humans have been reported (Britton, 1983; Cerda-Olmedo, 1989; Nishino, 1995).

Colony diameter. Average colony diameters of all five isolates of *C. militaris* after first, second and third weeks of incubation under light are given in Table 6. Radial growth of the colonies was more rapid in media producing moderate mycelial density when compared with those producing poor or abundant mycelial density. Nutritionally-rich media promoted abundant mycelial density, but radial growth was slower, causing small colony diameters; whereas nutritionally-poor media caused low mycelial density as well as slow radial growth (Table 6). Radial growth, on the other hand, indicates the growth rate of hyphal tips. After one week under light incubation, radial growth showed positive relationship with the mycelial density, i.e., abundant mycelial density produced longer radial growths while poor or moderate mycelial densities produced shorter radial growths. But after two and three weeks, radial growth showed negative relationship with the mycelial density. Radial growth was longer in MA,

Table 6. Mycelial growth of *Cordyceps militaris* EFCC C-7159 isolates at different incubation periods under both light and dark conditions

Medium	Incubation periods in light			Incubation periods in dark		
	1st wk.	2nd wk.	3rd wk.	1st wk.	2nd wk.	3rd wk.
WA	12.2 ± 0.6[a]	31.3 ± 1.1	51.4 ± 1.1	24.2 ± 1.0	55.9 ± 1.5	69.0 ± 2.1
MA	18.0 ± 2.1	43.7 ± 3.8	68.0 ± 3.0	24.6 ± 1.4	57.7 ± 2.6	66.4 ± 2.0
CMA	11.9 ± 0.8	38.7 ± 3.6	60.7 ± 3.7	24.9 ± 2.2	61.8 ± 2.0	82.9 ± 2.0
PCA	17.7 ± 1.3	42.9 ± 4.2	65.0 ± 5.2	23.9 ± 1.4	66.6 ± 4.1	72.9 ± 2.5
MEA	19.7 ± 0.7	47.7 ± 4.4	66.3 ± 4.0	21.9 ± 1.4	51.3 ± 2.7	57.5 ± 2.0
V8	17.1 ± 2.9	41.1 ± 4.1	64.6 ± 5.6	23.2 ± 2.7	67.3 ± 2.1	76.6 ± 3.2
ES	17.8 ± 1.4	42.8 ± 2.8	67.7 ± 4.8	24.3 ± 2.5	59.2 ± 2.6	67.0 ± 2.1
OA	16.2 ± 3.2	43.0 ± 2.3	69.7 ± 4.7	23.6 ± 2.2	55.6 ± 0.6	67.0 ± 3.0
MYA	18.3 ± 2.3	42.1 ± 2.5	63.1 ± 3.4	17.9 ± 2.4	45.3 ± 3.7	54.4 ± 4.0
MM	18.2 ± 3.7	41.0 ± 4.2	63.7 ± 1.8	16.9 ± 1.5	51.0 ± 3.8	58.6 ± 4.2
YpSs	16.4 ± 0.9	45.1 ± 2.4	74.1 ± 3.9	23.9 ± 2.0	59.3 ± 3.2	76.1 ± 2.5
MPDA	19.6 ± 1.4	47.1 ± 4.2	72.0 ± 2.8	21.3 ± 1.1	55.9 ± 1.5	69.6 ± 1.4
PDA	18.5 ± 2.4	42.9 ± 2.2	68.7 ± 2.5	24.3 ± 1.2	64.3 ± 1.0	77.5 ± 2.2
MCM	23.7 ± 0.8	50.9 ± 1.9	75.7 ± 4.5	21.5 ± 1.5	58.6 ± 2.4	72.6 ± 3.0
SMA	23.1 ± 0.6	49.0 ± 1.1	70.6 ± 1.5	19.9 ± 1.4	53.2 ± 2.1	55.6 ± 3.9
SDA	22.1 ± 0.5	47.1 ± 1.0	68.6 ± 1.6	19.0 ± 1.1	47.6 ± 1.5	55.0 ± 1.5
YMA	19.1 ± 1.9	42.6 ± 4.7	67.9 ± 3.0	16.6 ± 2.4	43.3 ± 2.0	54.7 ± 2.6
HA	20.6 ± 1.2	48.7 ± 0.7	78.0 ± 2.4	21.9 ± 1.6	56.0 ± 1.4	67.3 ± 2.6
CDA	16.6 ± 0.7	39.6 ± 1.6	59.6 ± 2.7	18.3 ± 2.6	50.6 ± 1.2	65.6 ± 3.4
CZYA	23.2 ± 1.6	43.0 ± 2.2	63.9 ± 4.1	20.6 ± 0.6	54.9 ± 2.4	64.2 ± 2.7
SMAY	17.6 ± 0.6	40.7 ± 0.6	62.1 ± 2.9	18.2 ± 1.0	45.3 ± 1.1	59.9 ± 2.2
SDAY	16.6 ± 1.7	39.9 ± 2.1	61.6 ± 2.5	19.6 ± 1.6	47.0 ± 1.5	62.0 ± 3.6

[a]Colony diameter was measured of all the five isolates of EFCC C-7159 in triplicate in each medium at every incubation periods under both light and dark conditions. The average colony diameter (mm ± S.D.) was calculated for all the isolates at each incubation period for each medium under both light and dark conditions.

PCA, MEA, V8 and ES, which produced poor to moderate mycelial density when compared with SMAY and SDAY, which produced abundant mycelial density. Thus, in *C. militaris*, radial growth rate had no relationship with mycelial density after long incubation periods. Good radial growths were observed in YpSs, MPDA, MCM, SMA and HA. On the other hand, the least radial growth was shown on WA.

Also under dark incubation, radial growth showed negative relationship with mycelial density. Nutritionally rich media such as SMA, SDA, YMA, SMAY and SDAY produced abundant mycelial density but shorter radial growth, when compared to nutritionally poor media (Table 6, Fig. 2). Surprisingly, WA produced higher radial growths than most of the other media. Also, when compared to light incubation, dark incubation induced more rapid radial growths (Table 6). Hence, the inhibitory effect of light on the growth of *C. militaris* could be observed in this study.

In fungi, composition of complete media varies from species to species, but most of them contain casein hydrolysate, hydrolyzed yeast nucleic acid, a synthetic mixture of water-soluble vitamins, and perhaps yeast extract or malt extract or both (Fincham *et al*., 1979). Based on the observed growth characteristics from the present study, it is suggested that SDAY, SMAY, CZYA can be regarded as nearly complete media for the mycelial growth of *C. militaris*.

In future, nutrition- and environment-dependent mutations of *C. militaris* can be studied by using a large number of single spore isolates. Since *C. militaris* shows heterothallism during *in vitro* fruiting (Shrestha *et al*., 2004, 2005), phenotypic characteristics of the isolates of this fungus can be utilized for the selection of superior isolates for higher fruit body production and for the isolation of bio-active compounds. Changes in phenotypic characteristics of the isolates of *C. militaris* during subculturing can also be studied in relation to fruiting nature or production of bio-active compounds.

Cordyceps species have been mostly found to be difficult to grow in artificial culture. In spite of that, much attention has been paid to produce their mycelia for the commercial production of active compounds. Knowledge on growth characteristics of other *Cordyceps* species might be useful to understand their growth requirements. The results of this study can be extended to grow medicinally important *Cordyceps* species *in vitro* for the mass production of their mycelial biomass. Further studies are also required to show the effect of culture medium on fruiting body production of *Cordyceps* species, including *C. militaris*.

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